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Method validation for bifenthrin emulsifiable concentrate and uncertainty calculation using gas chromatographic approach

Arif Nazir^{1*}, Javaid Iqbal¹, Munawar Iqbal¹, Mazhar Abbas² and Numrah Nisar³

Abstract

Background: Bifenthrin is the third-generation synthetic pyrethroid insecticide having an effective control on the pest of cotton, vegetable, and fruits. This study is focused on the validation of the test procedure for the quantitative determination of bifenthrin contents in the emulsifiable concentrate and measurement of uncertainty. The purpose of this validation procedure is to demonstrate that it is suitable for the intended use. This was determined by gas chromatography with a flame ionization detector (FID). The estimation was carried out on Shimadzu GC equipped with TRB-5 (95% dimethyl, 5% diphenyl polysiloxane) column using a nitrogen carrier gas.

Results: Different parameters of validation (precision, accuracy, linearity, specificity, selectivity, and robustness) were executed. All steps of method validation were performed, and its uncertainty is determined. The method is simple, selective, accurate, precise, cost-effective, and suitable. The validation parameters are based on harmonized guidelines on the validation of the analytical test method.

Conclusions: Pesticide formulation bodies can use this method for the qualitative and quantitative determination of bifenthrin different formulations. These data verify that the method is validated, and all results are in an acceptable limit. The method is developed on GC. Moreover, the analysis time is also short as compared to that of HPLC. The developed method is simple reliable and has a realistic approach.

Keywords: Pesticide, Method development, Validation, Gas chromatography, Robustness

Background

Pesticides are being employed at a large scale throughout the world for the control of pests and for the good yield of crops in the agriculture sector [1–7]. There are so many pesticide groups including organophosphates, organochlorides, carbamates, and pyrethroids. Among all the pesticides, pyrethroids are in good fame for application to control the pests. Pyrethroids have efficient control over pests like jassid, thrips, and whitefly [8–10]. The major sucking insect pests whitefly (*Bemisia tabaci* Genn), thrips (*Thrips tabaci* Lind), and jassid (*Amrasca biguttula* Ishida) are harmful to the cotton and are responsible for 40% damage of cotton [5, 11, 12]. It is

studied that pyrethroid sticks and activates the voltage-sensitive sodium channels of the heart, nerve, and skeletal muscle cell membranes in the nervous system of insects which ultimately proceed to death. Paralysis is the startling effect of pyrethroid which leads to death. Pyrethrins have a low level of toxicity in mammals and birds [13–15].

Bifenthrin, cypermethrin, deltamethrin, and permethrin are the examples of pesticides from group pyrethroids. Bifenthrin is the third-generation synthetic pyrethroid insecticide having an effective control on the pest of cotton, vegetable, and fruits.

Chemically, bifenthrin is 2-methylbiphenyl-3-ylmethyl (Z)-(1 RS)-cis-3-[2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethyl cyclopropane carboxylate. Bifenthrin has shown assurance in the pest control of vegetables [7, 16]. Figure 1 shows the structural formula for bifenthrin. Environmental

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Fig. 1 Structure of bifenthrin

Protection Agency (EPA) has registered the bifenthrin for ornamental and cotton pest control usage. It is one of the moderately stable active ingredients under sunlight conditions. Its toxicity level is oral rat LD 50 = 54 to 70 mg/kg. The mode of action of bifenthrin is through the central nervous system. Skin irritation effects of bifenthrin can last for 12 h.

Bifenthrin is a pyrethroid insecticide that is widely used to kill the insects [8]. There is effectual control of aphids, jassid, and whiteflies through bifenthrin 10 EC with 97.9%, 85.9%, and 86% mortality rate, respectively [17]. Bifenthrin is very effective against the malarial attack and inhabits the mosquitoes for blood-sucking activity [18–21]. Literature review showed that bifenthrin was analyzed and quantified through different techniques like GC-AED [22], hall electrolytic conductivity detector [23], GCMS [24], GC with ECD [25], HPLC [26], GCMS using 100% polydimethylsiloxane) fused silica capillary column [1], GC with ECD, and dimethylpolysiloxane column [9, 10, 12, 15, 27–30].

Ermer and Miller [31] reported that validation includes the process and steps that ensure the suitability of the intended method for a particular test. ISO 8402:1994 defines the validation as “Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled.”

Validation of the test method will make it reliable for testing. The confidence level for the results extracted

through a validated method is more than a non-validated method. Validation ensures the fitness for purpose. Method validation is a reliable process having different analytical steps for the trueness of any analytical test method for implementation onwards for testing. Internationally, it is recommended that the laboratory must take some evaluations that convince the dependability of the test method. Method validation and uncertainty calculation will help to determine the capacity of any test methods. Both of these are also recommended by ISO 17025 for any laboratory. So, both of these have solid references to make it applicable and practicable in any laboratory. Method validation includes suitability, specificity and selectivity, precision, accuracy, linearity, robustness, limit of detection, and limit of quantification [32–34].

Uncertainty is mandatory for any measurement which will ensure the reliability of that measured results. Uncertainty includes the involvement of all factors which may affect the test results up to defined value [35]. The main objective of this study was to perform method validation and to calculate uncertainty measurements.

Methods

All the chemicals and reagents employed in this study were of analytical grade and were purchased from Sigma Aldrich. The stock solution of bifenthrin is prepared by taking 0.11 g in 25 mL flask. Add 10 mL and 1% internal standard solution (ISS) followed by mixing and then

Table 1 Conclusive data with peak areas and standard deviation for system suitability

Sr. #	Peak area of ISS solution	Peak areas of active	Area ratio	Retention time (min)	Standard deviation	RSD	Theoretical plates	
							ISS	Active
1	1823681	1834648	1.006	8.68	0.0027	0.27%	17999.012	17890.002
2	1824790	1825198	1.0002				17999.012	17890.002
3	1824790	1833184	1.0046				17870.674	17580.582
4	1824720	1832931	1.0045				17333.248	17990.111
5	1824854	1825212	1.0002				17970.624	17480.597

Fig. 2 Linearity graph showing the relation of bifenthrin concentration and peak area ratio

filtration for injection into GC injector. 1% ISS is prepared by taking 1 g di-butyl phthalate (DBP) into a 100 mL volumetric flask and made up the volume with acetone solvent.

Sample preparation

0.97 g of emulsifiable concentrate pesticide formulation sample was taken into 25 mL volumetric flask. Ten milligrams of internal standard solution is added through a pipette. Mixed it well and sonicate for 1 min. The sample was then filtered through a 0.45- μ syringe filter, and after filtration, the sample was injected into GC.

A GC equipped with a flame ionization detector (FID) was used for the present research work. The separation

was achieved using TRB-5 (95% dimethyl, 5% diphenyl polysiloxane bonded and cross-linked phase) (0.25-mm ID, 30-m length, and 0.1- μ m film thickness). The carrier gas was nitrogen with a flow rate of 20 mL/min for 4.5 min and then increased to 25 mL/min for 9 min at the rate of 20 mL/min. The column temperature was 220 °C for 5 min and then increased to 260 °C for 9 min at the rate of 50 °C per min. Injector temperature and detector temperature were 260 °C.

Method validation

Suitability of the system is checked by injecting the same solution of active ingredient repeatedly more than one time; each and every time the peak area of the active

Fig. 3 Linearity graph showing the relation of bifenthrin concentration and peak area

Table 2 Bifenthrin responses summarized according to the increased concentration (linearity)

Sr. #	Weight (g) of bifenthrin in 10 ml ISS solution	Peak area of an active ingredient	Peak area of internal standard solution	Area ratio
1	0.06	1233374.13	1821854.20	0.677
2	0.07	1355674.55	1821564.00	0.7442
3	0.08	1484997.40	1821874.00	0.8151
4	0.09	1604702.84	1821884.77	0.8808
5	0.10	1724002.00	1821894.61	0.9463
6	0.11	1825131.00	1821804.00	1.0018
7	0.12	1948416.00	1821819.12	1.0695
8	0.13	2082691.41	1821834.13	1.1432
9	0.14	2224951.00	1821849.57	1.2213
10	0.15	2359950.41	1821864.17	1.2953
11	0.16	2500250.00	1821879.01	1.3723

ingredient was consistent with reasonable relative standard deviation. The linearity of the method is confirmed by injecting the solutions having increased concentrations in specific regular proportions. The specificity and selectivity of the method are tested. This was done by making the solutions having ingredients of test method and formulation separately in a unique way. Accuracy is the ability of the test method to conclude the results near the true value. Accuracy is verified after getting the analysis results of known increased concentrations (80%, 100%, and 120% of claimed active ingredient) [36–39]. The precision is the recovery of the results over and over again when each time tested. The robustness of the test

method is evaluated by changing different parameters of the test method. The limit of detection and limit of quantitation are calculated by signal to noise ratio [27, 40–42]. The uncertainty of the test method estimates using the Eurachem guide [43].

Results

Suitability and linearity

Five samples having a concentration of 0.11 g of the standard bifenthrin were injected repeatedly to check the response of the system. The response of the system is shown in Table 1.

Fig. 4 Representative gas chromatogram showing the specificity and selectivity of the method

Table 3 Data for accuracy and precision studies

Sr. #	Active ingredient added (w/w %)	Final Conc. of sample prepared (%) [spiked]	Active ingredient quantified (%) [found]	Recovery (%)
1	80	19.80	19.78	99.98
2	100	22.00	22.10	100.45
3	120	24.20	24.19	100.86
Precision data				
	Day 01 analysis results (W/W%)	RSD	Day 02 analysis results (W/W%)	RSD
1	10.99	0.577	10.99	0.577
2	11.00		11.00	
3	11.00		11.00	

The calibration curve was made of concentrations ranges from 0.06 g bifenthrin/10 ml ISS solution to 0.16 g bifenthrin/10 ml ISS solution (Figs. 2 and 3; Table 2).

Specificity and selectivity

During the testing, the acetone is used while during the formulation of bifenthrin, 10 EC emulsifier and xylene are used. Injecting solutions of each of these is prepared and injected into GC to check the response of all ingredients, and it was found that no interfering peaks were observed in the chromatograms during the testing with active ingredient peak area (Fig. 4).

Accuracy and precision

Accuracy is assessed by analyzing a sample with known concentrations and comparing the measured value with the true value as supplied with the material (Table 3). The precision of the method is checked by analyzing the samples in repetition. The standard weight taken was 0.11 g and then diluted up to 10 mL of internal standard solution, and a sample taken was 0.97 g ($\pm$ 0.005 g) which is diluted up to 10 mL through the same internal standard.

LOD and LOQ

Limit of detection (LOD) is the lowest amount of the active ingredient that can be detected, and the limit of

quantitation (LOQ) is the lowest amount of analyte that can be detected and can be quantified. The LOD is not necessarily that which can be determined and accepted with accuracy and precision. Through the signal to noise ratio, the LOQ and LOD are determined. The samples are injected to such extent that the signal to noise ratio was 3:1, and the amount of active sample responded to ratio 3:1 is LOD. On the other hand, the sample amount responded to a signal to noise ratio 10:1 is LOQ.

For robustness, the ability of the method is checked by doing small variations in the method parameters. The conclusive data acquired from different variations is summarized (Table 4) while uncertainty measurements are given in Table 5. Uncertainty can be expressed in both ways, i.e., positive and negative.

Discussion

Analytical method development and validation are an important process for pesticides. This is to ensure quality and safety. The analytical methods provide data for analytical problem sensitivity, accuracy, precision, and range of analysis. These requirements essentially are the specifications to analyze the desired analyte with certainty. Five samples having a concentration of 0.11 g of the standard bifenthrin were injected repeatedly to check the response of the system. The response of the system in terms of peak area, retention time, and theoretical

Table 4 Summarized data for robustness

Parameters	Results (w/w)	Results (w/v)	FAO ranges
Oven temperature 200 °C	10.78	9.81	9.00–11.00 (w/v %)
Oven temperature 220 °C	11.00	10.01	
Injector temperature 250 °C	10.90	9.92	
Injector temperature 270 °C	10.90	9.92	
Detector temperature 250 °C	10.99	10.00	
Detector temperature 270 °C	10.97	9.98	
Sample weight (0.8702 g)	10.97	9.98	
Sample weight (0.9702 g)	11.31	10.29	

Table 5 Uncertainty calculation

		Uncertainty Measurement					
TEST TITLE		Bifenthrin 10%w/v (11%w/w) Emulsifiable Concentrate					
TEST METHOD		Chemical Analysis of Bifenthrin					
S/N	Observations	Day 01	Day 02	SD (a)			
1	Reading 1 (w/w)	11	11.01	-			
2	Reading 2 (w/w)	11	11.00	-			
3	Reading 3 (w/w)	10.99	10.99	-			
6	Average	11.00	11.000	0.0024			
7	SD (r)	0.005774	0.01	-			
Maximum Standard deviation Value of Repeatability				0.01000			
Uncertainty of Repeatability - SD (r)				0.00577			
Standard Deviation of Mean of Reproducibility				0.00236			
Uncertainty of Reproducibility - SD (a)				0.00167			
Average value Reproducebility (R)				10.99833			
UNCERTAINTY MEASUREMENT (Budget)							
S/N	Sources of Uncertainty	Standard Uncertainty Using Distribution	Uncertainty	Type A/B	Value	Relative Uncertainty	Square of Relative Uncertainty
1	Repeatability	N/A	0.005773503	A	10.99666667	0.000525023	2.75649E-07
2	Reproducibility	N/A	0.001666667	A	10.99833333	0.000151538	2.29638E-08
3	Sample Weight	0.00005	0.00005	B	0.9702	0.000052	2.65594E-09
4	Standard Weight	0.00006	0.00006	B	0.11	0.000500	0.00000025
4	CRM	0.002886751	0.002886751	B	0.98	0.002945665	8.67694E-06
5	Glass ware	0.288675135	0.288675135	B	100	0.002886751	8.33333E-06
6		0.034641016	0.034641016	B	10	0.003464102	0.000012
7	GC (Oven)	0.0500000	0.0500000	B	260	0.000192308	3.69822E-08
	(Injector)	0.0500000	0.0500000	B	260	0.000192308	3.69822E-08
	(Detector)	0.0500000	0.0500000	B	260	0.000192308	3.69822E-08
							2.96725E-05
							0.005447246
Combined Uncertainty (Uc)		0.059910627	@	99 % CL	lower Limit of Analysis Result		9.00%w/v
Confidence Level (K)		3	=	99% CL			
Expanded Uncertainty (Ue)		0.18	@	99% CL	Upper limit of Analysis Results		11.00%w/v

plates were evaluated. The importance of equipment suitability cannot be neglected as it shows the availability of equal response on getting the analyte. The relative standard deviation (RSD) of the observed retention factor (RF) values is 0.27% which confirms that the system is suitable for analysis. These results were consistent. Linearity is the step of the validation that verifies the response of the detector against the subjected sample. The absence of linear response may be attributed to

equipment, extraction solvent, dilution, injection volume, or complex formation issues.

Measuring the samples with reference to accuracy, it is the closeness of the results that is accepted.

The response must be linear if the concentration is increased in the same pattern. During testing, acetone is used while during the formulation of bifenthrin, 10 EC emulsifier and xylene are used. Injecting solutions are prepared and injected into GC to check the response of

all ingredients. It was found that no interfering peaks were observed in the chromatogram during testing with an active ingredient peak area. Varying amounts of active ingredient (80%, 100%, and 120% of claimed active ingredient) are spiked. The precision of the method is checked by analyzing the samples in repetition. On day 1, standard and samples against these standards were injected to get results. Similarly, on day 2, freshly prepared standard solutions and sample solutions were injected to get the detector response.

The robustness defines the flexibility of the intended test method for validation. Different types of changes done in the test method as we changed the column temperature, detector temperature, injector temperature, sample temperature, etc. After changing the method parameters, there should be no prominent/effective change in the test result.

Uncertainty is the range in which the concluded value may resonate. Error expresses as a problem and can only be expressed (in statistical terms) as either positive or negative value, while uncertainty is expressed in both forms positive and negative [37, 44, 45]. Uncertainty from two types of sources is calculated and then merged into the final uncertainty budget. Type A was calculated through reproducibility and repeatability standard deviation which onwards used to measure standard uncertainty. Type B is calculated through different distribution laws like normal distribution, rectangular distribution, and triangular distribution law. Through the uncertainty budget, relative uncertainty is calculated. Combined uncertainty results through relative uncertainty which multiplied with coverage factor resulted in expanded uncertainty with a certain confidence level. The uncertainty value of the test method was calculated is 0.18%w/w with a 99% confidence level. The proposed method was found to be simple, swift, linear, accurate, precise, and robust for the determination and quantification of bifenthrin. Hence, the method could be easily and conveniently adapted for routine analysis.

Conclusions

The following conclusions are drawn:

Method validation was performed as per ICH guidelines. The method was validated for precision, linearity, robustness, accuracy, specificity, and precision. The data verifies that the method is validated, and all results are in an acceptable limit. The method is developed on GC. Analysis of the bifenthrin 10 EC can be possible on HPLC also, but it requires considerable amounts of solvents for sample dilution as compared to GC. Moreover, the analysis time is also short as compared to that of HPLC. The developed method is simple and reliable and has a realistic approach and could certainly and appropriately implemented for routine analysis.

Abbreviations

DBP: Di-butyl phthalate; EC: Emulsifiable concentrate; ECD: Electron capture detector; EPA: Environmental Protection Agency; FAO: Food and Agriculture Organization; FID: Flame ionization detector; GC: Gas chromatography; GC-EAD: Gas chromatography-electroantennogram detector; GCMS: Gas chromatography-mass spectrometry; HPLC: High-performance liquid chromatography; ID: Internal diameter; ISO: International Organization for Standardization; ISS: Internal standard solution; LOD: Limit of detection; LOQ: Limit of quantification; RF: Retention factor; RSD: Relative standard deviation; USA: United States of America

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Authors' contributions

AN contributed to the conception, design, interpretation of data, and preparation of the first draft. JI has performed all the experiments and was responsible for data acquisition. MI has proofread the draft of the work for the project and also helped in the interpretation of data. MA has helped in the revision of the manuscript. NN has proofread the revised manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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